THE PROCESS OF RECOVERY FROM THE FATIGUE OCCASIONED BY THE ELECTRICAL STIMULATION OF CELLS OF THE SPINAL GANGLIA.

By C. F. Hodge, Ph. D.

Experiments Upon Cats.

The two preceding chapters, of which the following is a continuation, appeared in this Journal in May, 1888, and May, 1889. In the first it was shown that stimulation of a nerve going to a spinal ganglion produced a marked change in the appearance of the ganglion cells, as seen under the microscope. This change was most pronounced in the nuclei, which might, upon stimulation of seven hours, lose forty per cent. of their bulk. The cells themselves, further, decreased little in size, but in many cases the cell protoplasm became extremely vacuolated, and as a third difference the nuclei of the cell capsule shrunk to a noticeable degree.

In the second paper the attempt was made by a series of experiments, in each case the nerve being stimulated in the same way but for a different length of time, to ascertain whether the amount of change in the cells was directly proportioned to the length of time the work was continued. In a general way this was found to be true. In all cases the nerve upon the right side of the animal was stimulated, while that upon the left was at rest. After the experiment the stimulated ganglion was examined, using its resting mate of the left side as a normal to compare with.

By this method it was found that the nuclei lost respectively upon stimulation of 1 hour, 2½ hours, 5 hours and 10 hours, 22%, 21%, 24.3% and 43.9% of their bulk.

The series of experiments which I propose now to describe had for its object a study of the recovery of spinal ganglion cells after fatigue. If the changes seen in the specimens, the shrunken nucleus, vacuolation of protoplasm, shrinkage of

the capsular nuclei, are, in truth, evidences of normal or physiological fatigue, a period of rest being allowed, recovery should take place. Accordingly, a series of cats were worked in exactly the same manner for the same length of time and allowed to rest, before excision of the ganglia, different lengths of time, in order to observe the process of recovery, if it took place, at different stages.

Method.

I find that in previous descriptions I did not devote enough space to the method employed. And as this has been still further perfected by experience and by the use of the best available apparatus, I wish, before considering the experiments, to describe the method in some detail.

In the first place, in order to compare one experiment with another, we must know that the stimulation used in both is the same. We must, therefore, have in the primary circuit, first, a galvanometer to give us the strength of current; second, a resistance box or rheocord of some sort to control any irregularities in the strength of the battery; third, an arrangement of some kind to make and break the primary circuit at regular intervals, since continuous stimulation is not employed; fourth, a signal to record the beats of the interrupter; and fifth, an ordinary induction coil. For the battery I have used in the last series of experiments three grove cells. A Weston's direct reading am-meter, reading from 0 to 15 ampères, was placed next the battery. From this it was possible to read off the strength of current at any time. Next this in the circuit was placed an ordinary resistance box with rheocord attached. This is quite necessary for exact work, as the battery was set fresh at the beginning of each experiment and increased in power for the first hour or so and then gradually weakened until the end of the five hours, during which the stimulation lasted. These variations could generally be compensated for by merely sliding the bridge of the rheocord. The interval of rest and stimulation was the same as that adopted for the last series of experiments, viz., 45 seconds rest alternating with 15 seconds stimulation. In my first experiments a key was placed in the circuit and the circuit was made and broken by hand.

I am glad to acknowledge to Dr. Lombard my indebtedness for a most serviceable little device which removes this irksome feature of the experiment. A small nickel clock forms the basis of the contrivance. It must be provided with a second hand. The glass face-cover and all the hands are removed, and upon the shaft of the second hand is fastened an eccentric zinc disc $2\frac{1}{2} \times 3$ cm. in diameter. In front of the clock is held by a post, properly placed, a lever of hard rubber 15 cm. in length; the longer arm of the lever, 8 cm., is between the post and the clock, so that this end, which is tipped with a small gutta-percha wheel, to reduce friction, will tilt back lightly upon the eccentric. The other arm of the lever carries two light copper wires tipped with platinum. The platinum tips, extending downward at right angles from the lever, dip into a glass mercury cup. Thus the motion of the eccentric upon the second shaft is made to tilt the lever in and out of the mercury cup every minute. By placing the cup upon the head of a screw, so that it can be raised or lowered at will, and by proper shaping of the eccentric disc, it is very easy to arrange it so that the circuit is made through the mercury in the cup 15 seconds and broken 45 seconds, which is the spacing of intervals desired. The whole is arranged upon a small board, into which are screwed two binding screws for convenience in joining up with the circuits. It is only necessary to connect this automatic make and break key with the circuit.

It was decided to use 20 stimuli per second, and this rate was obtained by loading the interrupting hammer attached to the induction coil. As this was apt to jar out of adjustment, I was compelled to take the record of the interruption by placing a signal in the circuit which should write its vibrations upon a smoked drum, under the tracing of a signal in circuit with a seconds clock.

By these means it was possible to control the stimulation apparatus very accurately. A half ampère, as read from the galvanometer, was used throughout the series. The automatic key gave regular intervals of 15 seconds stimulation, with 45 seconds rest. The beat of the interrupter was kept

at 20 per second. The secondary coil was of course kept at the same place in each experiment.

The animals used were kittens six to eight weeks old, and a word as to their preparation may not be out of place. Nothing was fed after the commencement of the experiment, but up to that time they were so well fed that it was thought a fast of even twenty-nine or thirty hours would not complicate matters seriously, if at all. In operating, the kitten is laid on a holder and gently brought under the influence of ether. When fully anæsthetized the skull is trephined at about the parietal eminence, and a slit is made through the *dura mater*. The trephine should be the smallest size, 5 to 7.5 mm. in diameter. With kittens it is possible to lift out a small piece of the bone at this place with the point of a knife blade, with generally less loss of blood than is occasioned by trephining. Now, holding the head with the left hand, with the thumb upon the vertex, the tip of the first finger upon the angle of the right jaw, the tip of the third upon that of the left jaw, introduce, with the right hand, through the opening in the skull, the blunt end of a 3 mm. glass rod, and aim it directly at the angle of the right lower jaw, the opening being invariably made in the left parietal bone. The probe will then strike the floor of the skull, having pierced the right optic *thalamus*, and the right *crus*. Work the probe across the floor of the skull about three mm., to either side of its first position, toward the right and also toward the middle line and withdraw it. Introducing the probe again, direct it forward as before, but down, aiming to pierce the left optic *thalamus* and the left *crus*. Take about one 3 mm. step with the end of the probe to right and left, withdraw the probe and close the skin over the wound. The aim of the operation is, of course, to destroy the sensory and motor tracts in the *crura*. Remove the ether and allow the animal to recover. If the operation has been successful the animal will evince no signs of pain or distress, but will remain as though quietly sleeping during the rest of the experiment. In some cases, however, the animal does show signs of restlessness for a few minutes after it recovers from the ether. These gener-

ally pass off very soon and give place to the condition of quiet desired.

The next step is to get the electrodes over the desired nerves, in this case the nerves of the right brachial plexus. Turning the animal upon its back, expose the external pectoral muscles by an incision in the skin about two inches long midway between the *sturnum* and *axilla*. Cutting through the external and internal pectoral muscles will now expose the subclavian artery and vein, and just underneath these can be plainly seen the nerves of the brachial plexus. In order to prevent hemorrage, I always take the muscles up with a double row of ligatures and make the cut between them. Free the plexus of fat for a short distance and separate it from the subclavian vessels, and, not including these, slip over it from behind a two tined platinum electrode.[1] Thus the current is made to pass through the nerves obliquely.

The stimulation may now begin. The nerves are not divided, and every muscle of the right fore limb should contract. This, in fact, is an important test of the proper working of the apparatus. If all the motor nerves are stimulated, and are conducting the impulses properly to the muscles, there is every reason to think that the sensory nerves are also conducting their impulses centrally to the cells of the spinal ganglia.

The animal is now carefully tended while the stimulation proceeds. The temperature is frequently taken, and heat applied or removed as the case demands. Respiration and pulse are watched. Lymph is apt to collect in the *axilla* about the electrodes, and should be frequently wiped up with absorbent cotton. The skin is drawn together over the wound and held with a clamp, and the wound is further protected with an ample pad of cotton.

In this series of experiments, the stimulation was continued for five hours in each case. At the end of this time the animal

[1] The electrode first used was an ordinary platinum electrode such as was used to stimulate a muscle-nerve preparation. Thinking that it would be better to have the platinum tips guarded, I made an electrode by letting heavy copper wires into deep grooves in a strip of gutta-percha. Platinum wires were soldered to these, and lie half-exposed in shallow grooves upon the inner side of each of two fork-like prolongations of the gutta-percha.

is gently removed from the board, wrapped up and laid in a warm place, where it is left to sleep the desired length of time. When this has expired, the work of removing the ganglia is begun. This is done as quickly as possible. A single cut with a scalpel severs the medulla at the *foramen magnum*. The skin is opened along the dorsal median line, some of the muscles cut away, and, with a pair of slender pointed bone forceps, the arches of the *vertebræ*, from the fifth cervical to the second thoracic, are removed, care being taken not to injure the cord or spinal nerves. A preparation is thus obtained which should resemble, so far as the brachial region is concerned, the plate upon page 374 in Wilder and Gage's *Anatomical Technology*. I have always taken the additional precaution before removing the electrodes of tying a ligature around the nerves included by the tines of the electrode, so that I may be sure to note the fact, if any important branch of the plexus has escaped stimulation.

In this series I have used for study only the ganglia of the first thoracic and eighth cervical pairs, and of these the first thoracic pair is dropped into about 5 cc. of 1% solution of osmic acid, the eighth cervical into saturated mercuric chloride solution at 40° c. The ganglia should be in their respective killing solutions within five minutes from the time the animal is killed. My own practice has been to leave them in both of these fluids for four hours, after which they are carried through the remaining processes in the usual manner. Minor points of technique are, however, not essential, so long as this one point is observed, viz.: *that the two ganglia to be compared are carried through all processes of preparation absolutely together, from the body of the animal to the microscope slide.* We then have under the microscope, side by side, sections of both ganglia of the same pair, one of which has been experimented with, while the other remains as a normal with which to compare it.

The method of investigation from this point on is the same as that employed in the former series of experiments. The nuclei and cells are measured microscopically, the longest and shortest diameter of each being taken. The mean of all the measurements for the worked or normal nuclei is taken as

the diameter of a sphere. As the nuclei are very nearly spherical, this sphere may be conceived to represent the average bulk of the nuclei in the set, from which the average diameter is obtained. And a comparison of the average sphere of one set with that of the mate to the set gives in a crude, but in the most exact way yet attained, an idea of the quantity of change produced by the conditions of the experiment.

As there is often reason to distrust averages, I will give the actual measurements as they occur in my notes for Cat 17. The measurements were made with a Zeiss eyepiece micrometer ruled to $\frac{1}{1}$ micron. divisions (eyepiece, 8; objective 4.0 mm. $\times$ 500), hence each division equals $2\frac{2}{5}$ μ. They are given as they were read, in units of the micrometer eyepiece.

CAT 17.

Measurement of the diameter of the Nuclei.

After 5 hrs. Stimulation and 0 hrs. Rest.		Normal.	
Diameter.	Number of Measurements.	Diameter.	Number of Measurements.
8.5	3	9.	3
8.	1	8.5	6
7.5	4	8.	28
7.	17	7.5	17
6.5	17	7.	61
6.	48	6.5	29
5.5	30	6.	33
5.	44	5.5	12
4.5	14	5.	11
4.	17		200
3.5	4		
3.	1		
	200		
200 cells measured, average diameter for the set, 5.39.		200 cells measured, average diameter for the set, 6.83.	

The above is sufficient to show that the mean in these diameters is a fair average. The measurements stand in about equal numbers above and below it in both cases. The diameters of the normal nuclei are throughout larger. The largest nuclei being found among the normal cells, and the smallest among the stimulated cells.

The results of this whole series of experiments may be seen at a glance from the following table:

Series to show the Influence of Rest.

Right brachial plexus of each stimulated in the same manner for five hours.

		Nuclei.		Cells.
	Rest.	Mean diameter of Nucleus in μ.	Shrinkage in per cent.	Mean diam. in μ.
Cat 17.	0 hrs.	16.40 Left, normal. 12.93 Right, stimulated.	48.8	57 52
Cat 16.	6.5 hrs.	16.70 Left, normal. 15.09 Right, stimulated.	26.	56 54
Cat 21.	12 hrs.	16.34 Left, normal. 14.73 Right, stimulated.	26.	55 51
Cat 19.	18 hrs.	17.08 Left, normal. 16.03 Right, stimulated.	18.	56 55
Cat 18.	24 hrs.	17.01 Left, normal. 17.11 Right, stimulated.	+ 2.	58 58
From another series.[1]				
Cat 7.	Normal.	14.20 Left. 14.54 Right.	+ 6.9	

[1] Am. Jour. Psy. May, 1889, p. 395.

In this series the stimulation was severe. It must be remembered that during the period of work, so-called, the stimulation is applied for only 15 seconds each minute. Five hours, therefore, of stimulation represents only one hour and a quarter actual working of the cells. But in this short time the change is marked, as is shown by a shrinkage of 48.8 per cent. in the nuclei of the side stimulated. The cells, as before, shrink little, and the cell protoplasm exhibits considerable vacuolation.

The quantities expressed in the above table, while they show that the nerve cells do gradually recover from the effects of fatigue, tell nothing concerning the process of recovery. The table is, in fact, but a poor expression of even the amount of change. In the first place it is impossible to measure accurately the irregular and jagged outline of the worked nucleus. Our practice has been to measure to the

tips of the jagged points into which the nucleus is prolonged; and this would evidently tend to make the computed bulk larger than the actual volume. In the second place, the quantities in the table are averages, whereas for our purpose extremes are most interesting. In a study of the cells of a worked ganglion, we see some nuclei which are not affected at all; and this we should expect because it is impossible to stimulate all the nerve fibres going to a ganglion without disturbing its blood supply. We next find nuclei which are slightly worked. In their even outline there may be here and there only a slight indentation, with here and there a vacuole in the cell protoplasm. These nuclei may have shrunken 5 or 10 per cent. And so we pass, by all degrees of difference, to the cells which show extreme changes. And here the cell protoplasm is riddled with vacuoles and the nucleus has skrunken to a densely staining speck, which must have lost 75 to 80 per cent. of its original volume.[1]

To trace the process of recovery in a nerve cell from its condition in fatigue to that in the resting state, the ideal thing would be to watch a living nerve cell continuously for the required length of time. For the present, however, we have only the prepared specimens taken so as to give us presumably five steps in the process. The chief interest for us at present attaches to the nucleus.

To begin with, we find the normal nucleus a round or oval body with an average diameter, in the cat, of about 16μ. The outline is even and sharply defined. In the nucleus, generally near the center, is a single (for the spinal ganglion cells of the cat) round nucleolus. Beside the nucleolus, the normal nucleus, as seen in a section, has generally from one to four or five rather coarse granules or agregations of smaller granules, and is spun through with a fine reticulum. On the whole it appears clearer than the protoplasm of the cell; as though the greater part of its contents were unstained. This appearance it was that gave rise at first to the idea that the nucleus was a large vescicle or vacuole in the midst of the densely granular protoplasm.

[1] See plate Am. Jour. Psychology for May, 1889, p. 402.

As the cell is worked, the nucleus gradually loses its sharp outline and at the same time becomes clouded and filled with darkly staining granules. Two opinions are possible here: either that the granules already present in the nucleus and its reticulum, being drawn closer together in shrinking, give it a darker appearance, or that new granules are formed in the nucleus. My own observations incline me toward the latter view. The stimulation is continued, and the nucleus shrinks smaller and smaller, and becomes so dark in osmic acid specimens as to be hardly distinguishable from the almost black nucleolus. Whether the extreme limit of fatigue has been reached in any of the cells examined, is of course impossible to say; but if recovery can be taken as a sign of normal action, then the fatigue occasioned by working for five hours, as above described, is not abnormal or pathological.

The process of recovery is in general the reverse of that of fatigue. The nucleus and cell gradually return to the normal appearance. At the end of six and a half hours the cell protoplasm has apparently almost or quite recovered. Vocuolation is not observable in the specimen which has rested this length of time, or in any specimens which have rested for a longer period. The nuclei, however, although they have gained much in size, retain, to a marked degree, their dense stain. The process of recovery in this respect is not entirely completed in all the nuclei which have rested for 24 hours; it is still possible to find a few large but densely stained nuclei.

A study of the ganglion cells after long periods of complete rest has brought out a point of interest to the general histology of the nervous system as well as to the special subject in hand. An appearance, often noted in nerve histology, has hitherto complicated all our experiments. This is the fact that individual cells in the same ganglion present such great histological differences. Ranvier[1] calls attention to this fact

[1] Ranvier, Traité d' Histologie, Paris, 1889, p. 802. "How is it that a little spinal ganglion, placed in a solution of ammonium bichromate, all the elements of which are therefore submitted to the same influences, contains side by side cells modified in a manner so widely different? This is a fact which we cannot yet explain; but, upon which we must insist, because we see it repeated in the spinal cord, the cerebrum, the cerebellum, etc.; that is to say, in all organs containing ganglion cells."

and shows that it cannot be due to the action of the reagents, but must be attributed to some differences between the cells themselves. So in my own experiments, even in sections of normal ganglia, I invariably find a few cells which have all the appearances of being worked. The number of these in the normal ganglia varies, but may reach 5 to 10 per cent., while in the stimulated ganglia they often exceed 90 per cent. My supposition in such cases was that some of the ganglion cells had been more or less fatigued by the normal activity of the animal. But this was merely supposition. It might also have been supposed that these cells were in process of degeneration. After we have wrapped an animal up in cotton batting, however, and laid it in a warm chamber at constant temperature for twenty-four hours, its brain having previously been destroyed so that it makes no voluntary movements, after scarcely a sensory impulse has disturbed the cells for that length of time, we find, as we might naturally expect, all the cells in the most perfect resting state. The cells appear uniformly full, and not a single shrunken nucleus can be found. The nuclei, in fact, appear larger, rounder, and clearer than in any specimen I have hitherto examined. It would seem quite possible, then, that the differences between ganglion cells, seen in sections from the same specimen, may be due to the phase of nutrition or of functional activity in which each of the cells happened to be when it died or was killed by the reagent.

We have, in the foregoing, materials from which to construct a curve that may provisionally, at least, be taken to represent the process of fatigue and recovery in the cells of the spinal ganglia. Whether these results are applicable to the fatigue of nerve cells in general does not concern us at present. And whether the action of the nucleus may be fairly considered as an index of the whole process is open to question. But we have shown that this shrinkage of the nucleus is directly proportional to the duration and also to the strength of stimulation, and in general inversely proportional to the length of the period of rest. It is the only index we have at present, and we may be permitted to use it with the understanding that the curve obtained is entirely provisional.

The curve of fatigue for a muscle is generally described as a straight line, which falls more or less rapidly according to its load and the strength and frequency of the stimuli applied to it. That of a nerve fibre has been shown, for short intervals at least, to be a straight line which remains parallel to its base line; *i. e.*, within physiological limits a nerve fibre is not susceptible of fatigue.[1]

No curve representing the fatigue of the nerve cell, drawn directly from observation of the cell itself has hitherto been obtained. The nearest approach to this is perhaps to be found in such work as Mosso has done for the fatigue which manifests itself in voluntary muscular contractions.[2] If the curve which Mosso obtains can be taken to express the fatigue of the brain cells; we may say that the nerve cell tires rapidly at first, then very slowly, or possibly holds its own for some time, and finally falls quite rapidly again to a condition of complete exhaustion. If now we plot the percentages given in the table for a fatigue series (this JOURNAL, II, May, 1889, p. 395), we find a curve quite similar to some of the curves obtained by Mosso.

We have from the table above referred to, slight stimulation, for 1 hour, 2½ hours, 5 hours, and 10 hours, causing a shrinkage in the volume of the cell nucleus of respectively 22 per cent.; 21 per cent.; 24.3 per cent., and 43.9 per cent. This is represented to the eye by the dotted line in Fig. 1.

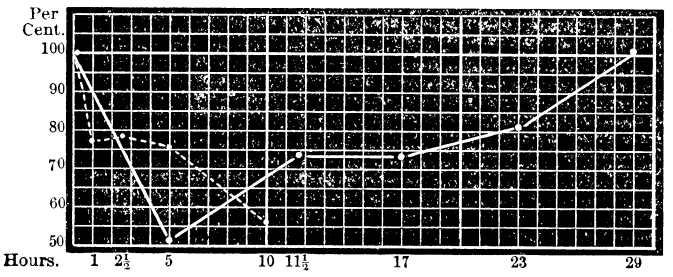

FIG. 1.

[1] H. P. Bowditch. On the Nature of Nerve-force. Jour. of Physiol., Vol. VI, p. 133.

[2] A. Mosso, Les lois de la fatigue, étudiées dans les muscles de l'homme. Travaux de Lab. de Physiol., de l'université de Turin, 1889. See plates, pp. 178, 185, 186.

By the continuous line of the figure is represented the process in the series of rest experiments, in which five hours of severe work has caused a shrinkage in the nucleus of 48.8 per cent.; recovery taking place as indicated by the second part of the curve. The curve of recovery in this instance is seen to rise quite rapidly at first, then more slowly, and again more rapidly to the normal.

I have already said that these curves are only provisional. In fact, they are introduced with the purpose of showing that in some degree they cannot be relied upon, rather than of attaching any permanent value to them. A most important factor in the shaping of these curves has hitherto been entirely ignored.

We have been endeavoring from the first to demonstrate the changes which occur in the normal functional activity of the nerve cell. That the changes already described do relate to the normal and not to the pathological action of the cells is proved by the facts that no pathological condition, which can affect the cells of the spinal ganglia, has been introduced into the experiments; that the changes in the cells are proportional to the severity and duration of their stimulation; and that the changes accompanying recovery are so far as we can see the natural reverse of those occasioned by fatigue. If, then, these changes are normal, why should there not be a rhythmic curve of rest and activity demonstrable in the normal activity of the animal. No more fundamental rhythm exists, in either physiology or psychology, than that of activity and rest, sleep and waking. And this rhythm, if not entirely dependent up on the condition of the nervous system, is, to say the least, very intimately connected with it. And if, as we know, marked changes are demonstrable in the normal activity of the cells of glands during hunger and digestion, (again rest and activity) why should not changes capable of demonstration occur in the rhythm of normal activity and rest in the cells of the nervous system?

If such a rhythm exists in the cells of the spinal ganglia, it is evident that such curves as we have obtained may be profoundly influenced by it. A stimulation of five or ten hours is physiologically a trivial matter compared with a fundamental rhythm which has become through generations an established fact in the economy of an animal species, and if the

changes in such a rhythm are similar to those which we have demonstrated by means of artificial stimulation, then clearly our results in each case have been *resultants* between the influence of our stimulation and the tendency of the rhythm at the time. Such considerations necessitate a study of the normal rhythm of sleep and activity in the animal employed. To this end I have kept under constant observation for a week a half-grown kitten similar to the ones used in my experiments. The sleep of such a kitten depends largely upon the amount of food given to it. When fed to repletion, it would sleep as much as eighteen hours a day; and even when sparingly fed, slept twelve and one-half or thirteen hours. It seemed to be able to sleep equally well day or night. In fact it will probably be necessary to study the next phase of our subject in some animal which has a more pronounced daily rhythm than that found in the cat.[1]

In no animal is this daily rhythm more constant or better developed than in our day birds. I have already made sections of the spinal ganglia of pigeons and English sparrows, taken at morning and night, and so far have found that the ganglion cells of these birds killed at night do show changes exactly similar to those produced in the cells of cats and frogs by artificial stimulation. The differences, further, between the morning and night cells are much more marked in English sparrows upon a cold and snowy day, for example, than I have been able to produce by the most severe electrical stimulation. The results of these experiments are, however, reserved for the present.

To conclude, then, we have as the result of the above series of experiments the following facts:

First, that spinal ganglion cells of kittens do recover from the effects of electrically stimulating the nerve going to them.

Second, that this recovery is a slow process. It is not complete after a rest of 18 hours; but is found to be about complete after a rest of 24 hours.[2]

[1] It will be noted that if the cat has no marked daily rhythm of rest and activity, then our curves are more probably correct.

[2] I have purposely omitted any attempt to discuss in this place the literature bearing upon the subject. This I hope to do in some more appropriate place; a general discussion of the literature touching the similar stimulation of gland and muscle cells, it has seemed, would necessitate the introduction of minute details of remote interest to psychology.